

Serotonin transporter activity of imidazolidine-2,4-dione and imidazo[2,1-*f*]purine-2,4-dione derivatives in aspect of their acid–base properties

Agnieszka Zagórska · Anna Czopek · Maciej Pawłowski ·
Małgorzata Dybała · Agata Siwek · Gabriel Nowak

Received: 14 September 2011 / Accepted: 4 November 2011 / Published online: 19 November 2011
© The Author(s) 2011. This article is published with open access at Springerlink.com

Abstract Affinities of arylpiperazinyllalkyl derivatives of imidazo[2,1-*f*]purine-2,4-dione and imidazolidine-2,4-dione for serotonin transporter and their acid–base properties were evaluated. The dissociation constant (pK_a) of compounds **1–22** were determined by potentiometric titration and calculated using pKalc 3.1 module of the Pallas system. The data from experimental methods and computational calculations were compared and suitable conclusions were reached.

Keywords Dissociation constant · Imidazolidine-2,4-dione · Imidazo[2,1-*f*]purine-2,4-dione · Serotonin receptor 5-HT_{1A} · Serotonin transporter

Introduction

Studies on major depression, anxiety, schizophrenia, mania, autism, obesity, and drug addiction have implicated the involvement of serotonergic (5-HT) abnormalities in these diseases. Serotonin acts via receptors which were classified into seven families (5-HT_{1–7}) and at least 14 different subtypes (Barnes and Sharp, 1999; Filip *et al.*, 2005; Hannon and Hoyer, 2008; Hoyer *et al.*, 2002; Pauwels, 2003). The level of 5-HT in central nervous system (CNS) and regulation of its neurotransmission are

connecting with serotonin transporter (SERT). This transporter is mediated extracellular uptake of serotonin from the synaptic clefts. The SERT protein belongs to the large family of transporters that are dependent on Na⁺ ions. Serotonin, Na⁺ and Cl[−] form a quaternary complex with the transporter before being co-transported across the plasma membrane, followed by counter transport of K⁺. At physiological pH = 7.4, serotonin is protonated and in the case of the SERT 5-HT accumulation was not affected by transmembrane pH differences (Rudnick *et al.*, 1989; Forrest *et al.*, 2007). Many drug molecules contain ionizable groups and hence penetrate across cell membranes, through pores and via active transport mechanism in a pK_a dependent fashion, therefore pK_a is an important factor on estimating the pharmacological behavior of drugs and their pharmacokinetic. This is particularly important in physiological systems, where ionization state will affect the rate at which the compound is able to diffuse across membranes and obstacles such as the blood–brain barrier (BBB) (Luan *et al.*, 2005; Manallack, 2007).

Since the early seventies until today, a large number of selective SERT inhibitors (SSRIs) have been described. However, only 5 products are currently marketed: fluoxetine (Prozac®), escitalopram (Lexapro®, Cipralex®), sertraline (Zoloft®), paroxetine (Paxil®), and fluvoxamine (Fevarin®). A major limitation of SSRIs and that extends to all other classes of antidepressants as well, is the 2–6 weeks delay in onset of therapeutic activity. This lengthy time to achieve remission is suspected to result from indirect activation of somatodendritic 5-HT_{1A} autoreceptors (Chaput *et al.*, 1986; Invernizzi *et al.*, 1992; Invernizzi *et al.*, 1996). The latest direction taken in antidepressant drug discovery has been to design ligands with multiple targets. Preclinical data obtained by co-administering a SSRI with selective 5-HT_{1A} antagonist, suggest that a single compound

A. Zagórska (✉) · A. Czopek · M. Pawłowski
Department of Pharmaceutical Chemistry,
Jagiellonian University Medical College, Medyczna 9 St,
30-688 Kraków, Poland
e-mail: azagorsk@cm-uj.krakow.pl

M. Dybała · A. Siwek · G. Nowak
Department of Pharmacobiology, Jagiellonian University
Medical College, Medyczna 9, 30-688 Kraków, Poland

combining SSRIs with 5-HT_{1A} antagonism should have a favorable therapeutic utility in the treatment (Artigas *et al.*, 1996; Ballesteros and Callodo, 2004; Adell *et al.*, 2005; Morphy and Rankovic, 2005; Millan, 2006). The most important class of 5-HT_{1A} receptor ligands are derivatives of arylpiperazine. Simple arylpiperazines are non-selective ligands for 5-HT receptor. The good selectivity and affinity for 5-HT_{1A} receptors show the majority of 4-substituted arylpiperazines. These derivatives contain a flexible aliphatic chain of different length (long-chain arylpiperazines, LCAPs), which connects the arylpiperazine fragment with second terminal pharmacophoric group (Lopez-Rodriguez *et al.*, 2002; Paluchowska *et al.*, 2007; Paluchowska *et al.*, 2005). For several years our attention has been focused on developing LCAPs containing a different amide/imide terminal fragment. In our earlier study a series of arylpiperazinylalkyl derivatives with a complex terminal part based on the purine moiety had been synthesized. Compounds with pyrimido- and imidazo-[2,1-*f*]purine-2,4-dione fragment have been tested in vitro for their 5-HT_{1A} and 5-HT_{2A} receptor affinities and were potent 5-HT_{1A} receptor ligands with K_i within the range of 5.6–278 nM and demonstrated lack of affinity for 5-HT_{2A} subtype (Zagórska *et al.*, 2009). The majority of imidazolidine-2,4-dione derivatives displayed high affinity for 5-HT_{1A} receptors (23–350 nM), and some of them exhibited significant affinity for 5-HT_{2A} receptors (Czopek *et al.*, 2010).

Continuing our research, we have been interested in affinities of arylpiperazinylalkyl derivatives of imidazo [2,1-*f*]purine-2,4-dione and imidazolidine-2,4-dione (hydantoin) for SERT and their acid-based properties presented as dissociation constant (pK_a) values. The aqueous ionization constant of a molecule is denoted by its pK_a values, where this constant is equivalent to the pH at which a given ionizable group on the molecule is half-ionized. In search of the structure activity relationship, the correlation with biological activity data with received pK_a values, was done.

Methods and materials

Chemistry

The chemical structures of derivatives of the imidazo [2,1-*f*]purine-2,4-dione and imidazolidine-2,4-diones investigated are listed in the Table 1. The hydrochloride salts of investigated compound 1–22 of the analytical purity (mp, TLC, elemental analysis) were used for the potentiometric titration.

Compounds 1–12 were obtained in the cyclocondensation reaction of 7-acetic-8-bromotheophylline aldehyde,

7-acetonil-8-bromotheophylline, and 7-phenacyl-8-bromotheophylline, with double amount of appropriate arylpiperazinylpropylamine, in boiling 2-methoxyethanol (Zagórska *et al.*, 2009). Whereas initial spirohydantoin were prepared from appropriate ketone by the Buchere–Berg reaction with modification described by Goodson *et al.* (1960). Compounds 13–22 were obtained by a two-step procedure involving alkylation of hydantoin at N3 position and condensation of intermediates with the substituted arylpiperazine or tetrahydroisoquinoline. The synthesis and physicochemical properties are described elsewhere (Zagórska *et al.*, 2009; Czopek *et al.*, 2010). The synthetic procedures and physicochemical data of compounds 16, 17, 21, and 22 have not been published yet.

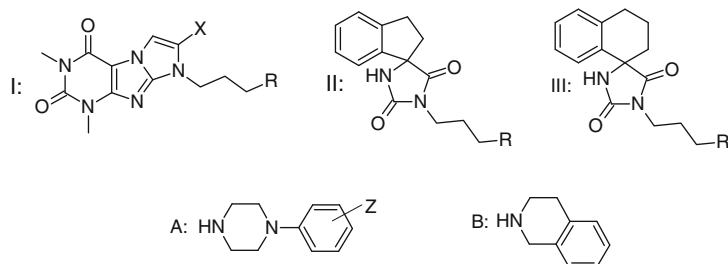
Pharmacology in vitro

The assay was performed according to the method of Owens *et al.* (1997) with slight modifications. [³H]-Citalopram (spec. act. 50 Ci/mmol, NEN Chemicals) was used for labeling 5-HT-transporter. Rat cerebral cortex was homogenized in 30 volumes of ice-cold 50 mM Tris–HCl containing 150 mM NaCl and 5 mM KCl, pH = 7.7 at 25°C and centrifuged at 20,000×*g* for 20 min. The supernatant was decanted and pellet was resuspended in 30 volumes of buffer and centrifuged again. The resulting pellet was resuspended in the same quantity of the buffer and centrifuged third time in the same conditions. 240 µl of the tissue suspension, 30 µl of 1 nM [³H]-citalopram, and 30 µl of the analyzed compound or 30 µl of 1 µM imipramine (displacer) were incubated at 22°C for 1 h. The concentrations of analyzed compounds ranged from 10^{−10} to 10^{−5} M. Incubations were terminated by vacuum filtration over Whatman GF/B filters and washed 5 times with 200 µl of ice-cold buffer. Radioactivity was measured in a MicroBeta TriLux– liquid scintillation counter (Perkin Elmer). All assays were done in duplicates.

Radioligand binding data were analyzed using iterative curve fitting routines (GraphPAD/Prism, Version 3.0 – San Diego, CA, USA). K_i values were calculated from the Cheng–Prusoff equation (Cheng and Prusoff, 1973). The results of in vitro binding studies (pK_i) of the compounds (1–22) are shown in Table 1.

Measurement of pK_a

The pK_a measurements were determined by potentiometric titration (alkalimetric), using a Compact Titrator Mettler Toledo G21 equipped with an integrated burette drive, and combined glass electrode DGi115-SC, compact rod stirrer, and 20 ml burette. Titrator was pre-programmed with standard tried-and-tested methods and calculations. The pH

Table 1 The structures of compounds **1–22**, their SERT activity (pK_i), experimental and theoretical pK_a values

Compd	Core	X	R	Z	pK_i [SERT]	Exp pK_a	pK_a Pallas
1	I	H	A	H	6.35	8.09	9.29
2	I	H	A	2-OCH ₃	6.95	8.19	9.29
3	I	H	A	3-Cl	7.53	8.35	9.20
4	I	CH ₃	A	H	4.95	7.55	9.29
5	I	CH ₃	A	2-OCH ₃	5.09	7.61	9.29
6	I	CH ₃	A	3-Cl	7.52	8.12	9.20
7	I	CH ₃	A	2,3-diCl	7.25	7.61	9.20
8	I	CH ₃	B	–	4.52	8.66	8.72
9	I	C ₆ H ₅	A	H	6.65	8.79	9.29
10	I	C ₆ H ₅	A	2-OCH ₃	4.69	8.44	9.29
11	I	C ₆ H ₅	A	3-Cl	6.72	10.61	9.20
12	I	C ₆ H ₅	B	H	5.61	10.41	8.72
13	II	–	A	H	5.96	10.48	8.95
14	II	–	A	2-OCH ₃	5.96	9.60	8.95
15	II	–	A	3-Cl	6.07	10.31	8.85
16	II	–	A	3-CF ₃	6.19	9.96	8.95
17	II	–	B	–	Nd	10.93	8.38
18	III	–	A	H	6.00	10.55	8.95
19	III	–	A	2-OCH ₃	6.01	10.32	8.95
20	III	–	A	3-Cl	6.04	10.80	8.85
21	III	–	A	3-CF ₃	5.62	11.08	8.95
22	III	–	B	–	5.40	10.90	8.38

electrode was first calibrated with buffers (pH = 7.00 and pH = 9.00). Sample (5×10^{-5} M) were prepared in water solutions (between 10–20 ml). Typically, more than 120 pH readings were collected for each titration. The deionized water used for the aqueous solution was twice distilled, degassed, and filtered with a Hydrolab Polska HLP5s System. The 0.0512 M sodium hydroxide solution were prepared from substances delivered by POCH. The buffers pH = 7.00 and pH = 9.00 used for calibration were obtained from Beckman Coulter. The pK_a were expressed as the mean of values of results from three titrations and are listed in Table 1.

The following equation was used for the calculation of the pK_a values:

$$pK_a = \text{pH} + \log \frac{2Ct - Ca}{Ca - Ct} \quad (1)$$

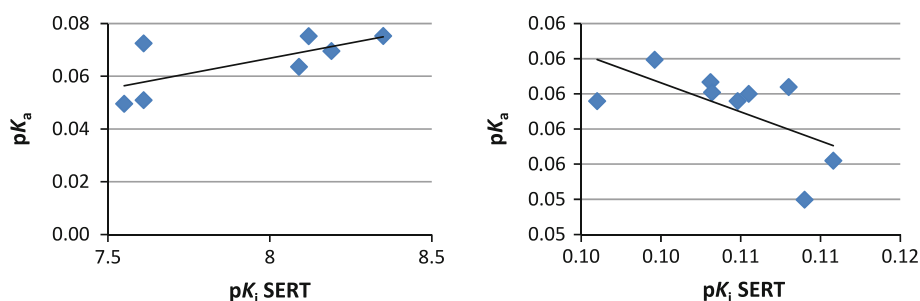
where Ct is a titrant concentration, Ca is a concentration of sample at each measured point.

Calculations

Calculations of pK_a were performed using Pallas 3.1 (CompuDrug Chemistry Ltd, 1995). Program applied logarithm, adapted after Hammett and Taft takes into account all necessary electronic, steric, and other effects and relies on an extended database of almost a thousand equations.

Regression analysis was performed using the Statistica for Windows program (Statistica for Windows, version 9, Statsoft Inc.2009). The significance level of the performed calculations was above 95%.

Fig. 1 Correlation between pK_a values and pK_i SERT of compounds **1–7** (left) and **13–22** (right)



Results and discussion

The library consisting of twenty two compounds was investigated. Based on their structural features, this library could be divided into two sublibraries: the first contained various arylpiperazinypropyl derivatives of imidazo[2,1-*f*]theophylline, and the second derived from imidazolidine-2,4-dione. Comparing the affinity for SERT obtained for imidazo[2,1-*f*]purine-2,4-dione and respective imidazolidine-2,4-dione analogues revealed higher activity in the first mentioned series. The most potent SERT ligands were compounds **3**, **6**, and **7** with pK_i within the range of 7.25–7.53, which were containing 2,3-dichloro or 3-chlorophenylpiperazine fragment in their structures. Compounds **1**, **2**, **9**, **11**, **12**, **15**, **16**, **19**, and **20** displayed moderate to very low affinity for the SERT (5.61–6.95), whereas other were practically devoid of any affinity.

Furthermore experimental dissociation constants for investigated compounds were determined. It seems that the basicity of the compounds mainly depends on two basic nitrogen atoms in piperazine ring. In general, compounds containing a substituent at phenyl ring electro-attracting atom (Cl) or group (CF_3) exhibited higher experimental pK_a values than unsubstituted ones. In addition, the replacement of arylpiperazine fragment with tetrahydroisoquinoline in respective compounds caused increase of pK_a values. The experimental pK_a values are in range from 7.55 to 11.08, the detail data are presented in Table 1. The ranges of predicted pK_a values of Pallas program are listed in Table 1. Unfortunately, the used program predicted no similar values to experimental pK_a and could not diversify the acid–base properties of closely related compounds. In order to obtain more detailed relationships between acid–base properties of investigated compounds and the affinity of tested compounds to SERT, QSAR studies were undertaken. It was found linear correlation between affinities for SERT (pK_i) and experimental pK_a values (Fig. 1) but ratio of determination was moderate ($R^2 = 0.48$ for sublibraries 1 and $R^2 = 0.38$ for sublibraries 2).

Summarizing, two compounds **3** and **6** (derivatives of imidazo[2,1-*f*]purine-2,4-dione) are potent dual ligands for SERT and 5-HT_{1A} receptor ($pK_i > 7.5$) and were classified to the further pharmacological studies. The obtained results

confirm that the applied potentiometric method is useful in characterization of the acid–base properties of closely related compounds contrary to values of pK_a predicted by Pallas program. There is no correlation between values of pK_a predicted by Pallas program and experimental. The moderate correlation between activity for SERT and pK_a , indicating that acid–base properties are one of the important factors, which could influent and modify the activity for SERT.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

- Adell A, Castro E, Celada P, Bortolozzi A, Pazos A, Artigas F (2005) Strategies for producing faster acting antidepressants. *Drug Discov Ther* 10:578–585
- Artigas F, Romero L, de Montigny C, Blier P (1996) Acceleration of the effect of selected antidepressant drugs in major depression by 5-HT_{1A} antagonists. *Trends Neurosci* 19:378–383
- Ballesteros J, Callodo LF (2004) Effectiveness of pindolol plus serotonin uptake inhibitors in depression: a meta-analysis of early and late outcomes from randomized controlled trials. *J Affect Disord* 79:137–147
- Barnes NM, Sharp T (1999) A review of central 5-HT receptors and their function. *Neuropharmacology* 38:1083–1152
- Chaput Y, de Montigny C, Blier P (1986) Effects of selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain. *Naunyn Schmiedeberg Arch Pharmacol* 333:342–348
- Cheng YC, Prusoff WH (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. *Biochem Pharmacol* 22(23):3099–3108
- Czopek A, Byrtus H, Kołaczowski M, Pawłowski M, Dybała M, Nowak G, Tatarczyńska E, Wesolowska A, Chojnacka-Wójcik E (2010) Synthesis and pharmacological evaluation of new 5-(cyclo)alkyl-5-phenyl- and 5-spiroimidazolidine-2, 4-dione derivatives. Novel 5-HT_{1A} receptor agonist with potential antidepressant and anxiolytic activity. *Eur J Med Chem* 45: 1295–1303
- Filip M, Frankowska M, Zaniewska M, Gołda A (2005) The serotonergic system and its role in cocaine addiction. *Pharmacol Rep* 57:685–700
- Forrest LR, Tavoulari S, Zhang YW, Rudnick G, Honig B (2007) Identification of chloride ion binding site in Na⁺/Cl[−] dependent transporters. *PNAS* 104(31):12761–12766

- Goodson LH, Honigberg IL, Lehman JJ, Burton WH (1960) Potential growth antagonists. I. Hydantoins and disubstituted glycines. *J Org Chem* 25:1920–1924
- Hannon J, Hoyer D (2008) Molecular biology of 5-HT receptors. *Behav Brain Res* 195:198–213
- Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. *Pharmacol Biochem Behav* 71:533–554
- Invernizzi R, Belli S, Samanin R (1992) Citalopram's ability to increase the extracellular concentrations of serotonin in the dorsal raphe prevents the drug's effect in the frontal cortex. *Brain Res* 584:322–324
- Invernizzi R, Bramante M, Samanin R (1996) Role of 5-HT_{1A} receptors in the effects of acute and chronic fluoxetine on extracellular serotonin in the frontal cortex. *Pharmacol Biochem Behav* 54:143–147
- Lopez-Rodriguez ML, Ayala D, Benhamu B, Morcillo MJ, Viso A (2002) Arylpiperazine derivatives acting at 5-HT_{1A} receptors. *Curr Med Chem* 9:443–469
- Luan F, Ma W, Zhang H, Zhang X, Liu M, Hu Z, Fan B (2005) Prediction of pK_a for neutral and basic drugs based on radial basis function neural networks and their heuristic method. *Pharm Res* 22(9):1454–1460
- Manallack DT (2007) The pK_a distribution of drugs: application to drug discovery. *Perspect Med Chem* 1:25–38
- Millan PJ (2006) Multi-target strategies for the improved treatment of depressive states: conceptual foundations and neuronal substrates, drug discovery and therapeutic application. *Pharmacol Ther* 110:135–370
- Morphy R, Rankovic Z (2005) Designed multiple ligands. An emerging drug discovery paradigm. *J Med Chem* 48(21):6523–6543
- Owens MJ, Morgan WN, Plott SJ, Nemeroff CB (1997) Neurotransmitter receptor and transporter binding profile of antidepressants and their metabolites. *J Pharmacol Exp Ther* 283(3):1305–1322
- Paluchowska MH, Bugno R, Bojarski A, Charakchieva-Minol S, Duszyńska B, Tatarczyńska E, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik E (2005) Novel, flexible, and conformationally defined analogs of gepirone: synthesis and 5-HT_{1A}, 5-HT_{2A} and D₂ receptor activity. *Bioorganic Med Chem* 13:1195–1200
- Paluchowska MH, Bugno R, Duszyńska B, Tatarczyńska E, Nikiforuk A, Lenda T, Chojnacka-Wójcik E (2007) The influence of modifications in imide fragment structure on 5-HT_{1A} and 5-HT₇ receptor affinity and in vivo pharmacological properties of some new 1-(*m*-trifluoromethylphenyl)piperazines. *Bioorganic Med Chem* 15:7116–7125
- Pauwels PJ (2003) 5-HT receptors and their ligands. *Tocris Rev* 25:1–10
- Rudnick G, Kirk KL, Fishkes H, Schuldiner S (1989) Zwitterionic and anionic forms of serotonin analog as transport substrates. *J Biol Chem* 264(25):14865–14868
- Zagórska A, Jurczyk S, Pawłowski M, Dybała M, Nowak G, Tatarczyńska E, Nikiforuk A, Chojnacka-Wójcik E (2009) Synthesis and preliminary pharmacological evaluation of imidazo[2, 1-*f*]purine-2, 4-dione derivatives. *Eur J Med Chem* 44:4288–4296